

ONE NEW SPECIES AND FIVE NEW RECORDS OF SOIL CILIATES (PROTOZOA, CILIOPHORA) IN CHINA

NING Ying-Zhi¹, LIU Han-Cheng¹, LIU Na¹, SUN Hong-Fei², ZHANG Chao¹, DU Hai-Feng¹, WANG Hong-Jun¹, MA Zheng-Xue¹

1. College of Life Science, Northwest Normal University Lanzhou 730070, China; E-mail: ningyz@nwnu.edu.cn

2. The Fifth Middle School of Fulaerji District of Qiqihar City, Qiqihar 161041, China

Abstract One new species and 5 domestic new records of soil ciliates collected from the Scenic Spots and Historic Sites of Maijishan and Xinglongshan National Nature Reserve, Gansu were described in this paper. They are *Keronopsis paradiackmanni* sp. nov., *Pseudochilodonopsis polyvacuolata*, *Tetrahymena pyriformis*, *Sterkiella caviola*, *Cyrtohymena tetracirrata* and *Euplates elegans*. The new species is characterized as follows: Size in vivo 180–320 μm $\times$ 75–110 μm ; elongated elliptical with both ends more or less broadly rounded; dorsoventrally flattened; usually 3–4 shortened elliptical macronuclei; 4 spherical micronuclei observed; 1 contractile vacuole. 6–8 frontal cirri; 5–8 buccal cirri; 3–4 rows of frontoventral cirri; 2 rows of ventral cirri, the right one reached the transverse cirri directly and the left one with 2 breaks ended above the transverse cirri; one left and one right row of marginal cirri, the right one extended on dorsal side anteriorly; 5–9 transverse cirri; no caudal cirri; 3 rows of dorsal bristles. AZM with 43–50 membranelles occupied about 1/4–1/3 of body length; paroral and endoral membrane curved with the former crossed over the latter about its anterior or posterior 1/3. Type specimens of new species were deposited in Institute of Ecology and Biodiversity, College of Life Science, Northwest Normal University, Lanzhou, China.

Key words Protozoa, Ciliophora, new species, new records, soil ciliates, China.

According to the classification system of Levine et al. (Levine et al., 1980), ciliates were classified under Phylum Ciliophora Doflein, 1901 of Subkingdom Protozoa Goldfuss, 1817. The key characters of Ciliophora are as follows: simple cilia or compound ciliature typical in at least one stage of life cycle, with infraciliature present even when cilia absent; two types of nuclei (macronucleus and micronucleus), almost without exception; asexual reproduction by transverse binary fission, also budding and multiple fission; sexual reproduction by conjugation, autogamy and cytogamy.

In terms of taxonomic and faunistic study, compared with freshwater and marine ciliates, less work has been carried out concerning soil forms in China. As a part of diversity survey on soil ciliates, one new species and five domestic new records of soil ciliates collected from the Scenic Spots and Historic Sites of Maijishan and Xinglongshan National Nature Reserve, Gansu were morphologically reported here.

1 Materials and Methods

Specimens were collected from the Scenic Spots and Historic Sites of Maijishan and Xinglongshan National Nature Reserve, Gansu (Table 1).

Sampling method according to Ning & Shen (1998) was adopted. The non-flooded Petri dish method (Foissner, 1992) was used for culture. Protocols for observation in vivo and protargol impregnation according to Song & Xu (1994) were employed in order to reveal

live and infraciliature features. Species identification was based on references of Curds (1975, 1983), Foissner & Didier (1981), Foissner (1997, 1998), Berger (1999), Berger & Foissner (1987), Song (2004), Jiang et al. (2008), Shen et al. (2008) and Fan et al. (2009).

2 Results

Keronopsis paradiackmanni sp. nov. (Table 2, Figs. 1–9)

Diagnosis. Size in vivo 180–320 μm $\times$ 75–110 μm ; elongated elliptical with both ends more or less broadly rounded; dorsoventrally flattened; usually 3–4 shortened elliptical macronuclei; 4 spherical micronuclei observed; 1 contractile vacuole. 6–8 frontal cirri; 5–8 buccal cirri; 3–4 rows of frontoventral cirri; 2 rows of ventral cirri, the right one reached the transverse cirri directly and the left one with 2 breaks ended above the transverse cirri; one left and one right row of marginal cirri, the right one extended on dorsal side anteriorly; 5–9 transverse cirri; no caudal cirri; 3 rows of dorsal bristles. AZM with 43–50 membranelles occupied about 1/4–1/3 of body length; paroral and endoral membrane curved with the former crossed over the latter about its anterior or posterior 1/3.

Type location. The specie was collected from the soils in Canglongling, hawthorn park in Quxi, larch forest in Dangchuan, the southern foot of Mount Shimenshan, Shimen Watershed and Wangjiadagou in

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Table 1. Sampling sites for each species.

	K. paradiECKmanni	P. polyvacuolata	T. pyriformis	S. caviola	C. tetracirrata	E. elegans
XJS			*			
WJX			*	*		
CLL	*		*	*		
QX	*		*			
DC	*					
SMS	*		*			
SMW	*	*				
WJDG	*					
TQ		*	*			
MJT		*		*		
PP	*		*			
HRS	*				*	
QU					*	*
CS						*
SP						*
BS						*
BG						*
GR						*

XJS-Xiangjishan; WJX-Wenjiaxia; CLL-Canglongling; QX-hawthorn park in Quxi; DC-larch forest in Dangchuan; SMS-the southern foot of Mount Shimenshan; SMW-Shimen Watershed; WJDG-Wangjiadagou; TQ-Tuqiao Watershed; MJT-Mujitan; PP-poplar plantation; HRS-Hippophae rhamnoides scrubland; QU-querceta; CS-clove scrubland; SP-spruce forest; BS-barberry scrubland; BG-birch grove; GR-grassland.

the Scenic Spots and Histosic Sites of Maijishan and poplar plantation and Hippophae rhamnoides scrubland in Xinglongshan National Nature Reserve, Gansu.

Type specimens. Type specimens were deposited in Institute of Ecology and Biodiversity, College of Life Science, Northwest Normal University, Lanzhou, China.

Etymology. The name paradiECKmanni recalls the fact that this species is similar to Keronopsis dieckmanni.

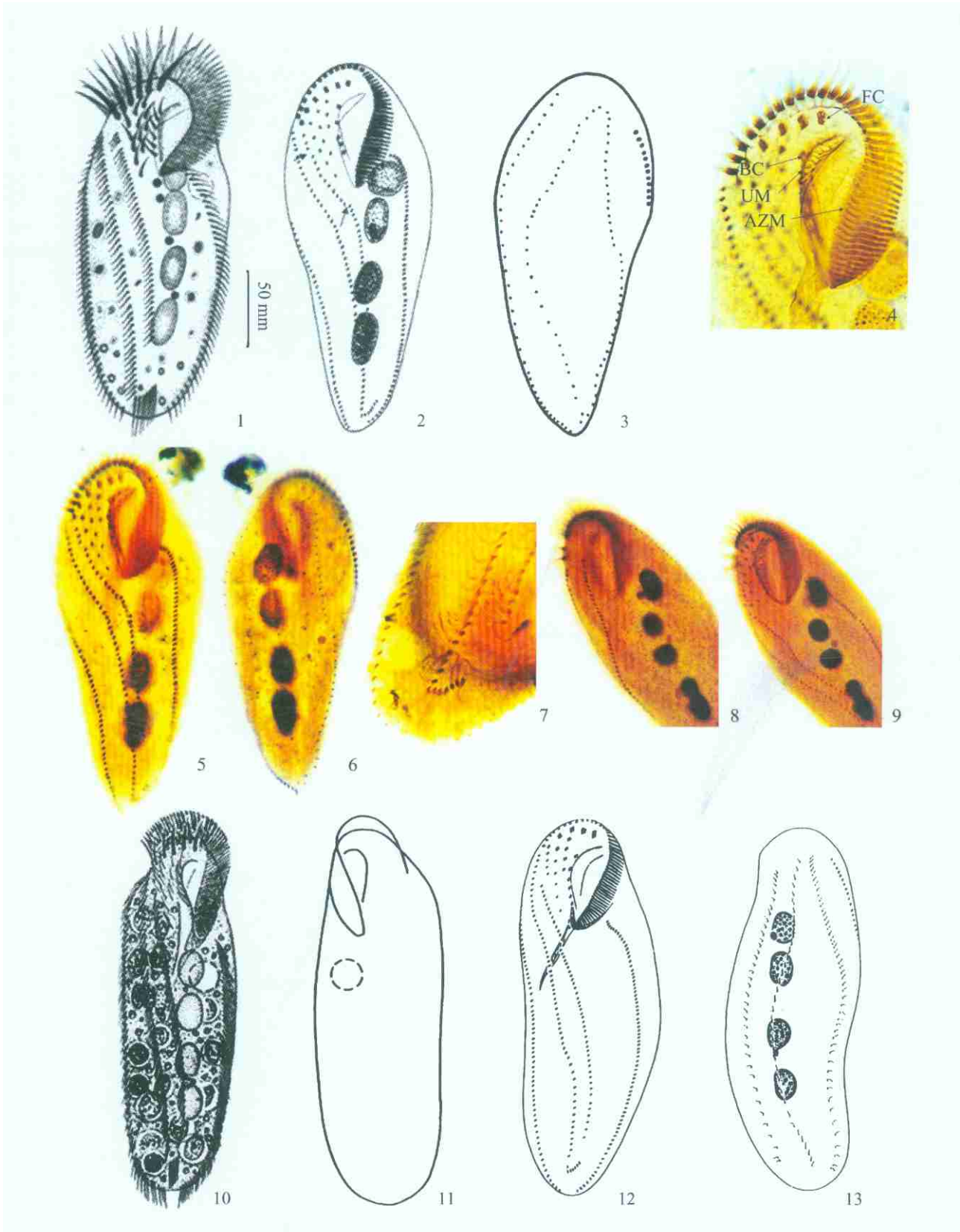
Description. Cell in vivo 180-320 μm ×75-110 μm

(n = 10); flexible, elongated elliptical with both ends more or less broadly rounded, but anterior portion slightly wider than posterior portion; dorsoventrally flattened, with ventral side nearly flat and dorsal convex; no cortical granules observed; colorless cytoplasm packed with yellowish fat globules; usually 3-4 elliptical macronuclear nodules one after another in the middle portion of cell slightly left of midline; 4 spherical micronuclei observed; single contractile vacuole distinctly above mid-body slightly left of midline.

Table 2. Morphometric characteristics of Keronopsis paradiECKmanni sp. nov.

Characteristics	$\bar{x}$	SD	CV	Min	Max	n
Body, length (μm)	235.00	49.58	21.14	180	320	10
Body, width (μm)	90.90	13.10	14.41	75	110	10
Macronuclear nodules, number	3.00	0.52	15.33	3	4	8
Micronuclei, number	4	0	0	4	4	2
AZM, length (μm)	66.50	9.36	14.08	56	80	10
AM, number	47.00	2.74	5.83	43	50	5
Frontal cirri, number	6	0.79	12.29	6	8	7
Buccal cirri, number	7	0.93	14.31	5	8	8
Frontal ventral cirri rows, number	4.00	0.52	14.65	3	4	11
Ventral cirri rows, number	2	0	0	2	2	10
Cirri in left ventral row, number	41.00	4.60	11.27	36	48	5
Cirri in right ventral row, number	48.00	5.67	11.86	42	56	5
Transverse cirri, number	7.00	0.94	13.43	5	9	10
Left marginal cirri row, number	1.00	0	0	1	1	10
Right marginal cirri row, number	1.00	0	0	1	1	10
Dorsal kineties, number	3.00	0	0	3	3	7

$\bar{x}$ -arithmetic mean; SD-standard deviation; CV-coefficient of variation; Min-minimum; Max-maximum; n-number of individuals observed.



Figs. 1-13. Morphology of *Keronopsis paradiackmanni* sp. nov. and *K. dieckmanni* from life (1, 10) and after protargol impregnation (2-9, 11-13). 1-9. *K. paradiackmanni* sp. nov. 1. Ventral view. 2, 5. Ventral view, arrows marked breaks in left row of ventral cirri. 4. Ventro-anterior view showing FC, FVC, BC, UM and AZM. 3, 6. Dorsal view showing dorsal bristles and the anterior of right row of marginal cirri extended on dorsal side. 7. Ventro-posterior view showing TC. 8. Dorsal side (perspective through ventral) showing macronuclei and micronuclei. 9. Ventral view showing macronuclei and micronuclei. 10-13. *K. dieckmanni* (from Foissner, 1998). 10. Ventral view. 11. Dorsal view of shape variant. 12-13. Infraciliature on both ventral and dorsal sides.

Six to 8 frontal cirri; 5-8 buccal cirri; 3-4 rows of frontoventral cirri with different patterns in different individuals, if 3 rows, 3-4-5 or 3-4-4 cirri arranged from the left row to the right one; if 4 rows, 3-4-5-5 or 2-3-3-4 arranged; 2 slightly spiral rows of ventral cirri extended diagonally from the posterior of the rightmost frontal cirrus to posterior portion of cell, the right one reached the transverse cirri directly and the left one with 2 breaks ended above the transverse cirri; one left and one right row of marginal cirri, the right one extended on dorsal side anteriorly; 5-9 transverse cirri; no caudal cirri; dorsal bristles arranged in 3 rows almost as long as body.

AZM with 43-50 membranelles occupied about 1/4-1/3 of body length; paroral and endoral membrane curved with the former crossed over the latter about its anterior or posterior 1/3.

Remark. As regards living morphology and ciliary pattern, our population should be assigned to the genus *Keronopsis*. So far this genus includes many species, among those, *Keronopsis dieckmanni* Foissner, 1998 resembled our form very much by having elongated elliptical body shape, ca. 50 adoral membranelles, 2 long ventral cirral rows, differentiation of enlarged frontal

cirri, two or more frontoventral cirral rows, one row of buccal cirri and transverse cirri, 3 dorsal kineties and 4 macronuclear nodules as well soil biotopes.

However, *K. paradiackmanni* differed distinctly from *K. dieckmanni* as follows: 1) different size, *K. dieckmanni* 165-245 μm $\times$ 48-91 μm , but *K. paradiackmanni* 180-320 μm $\times$ 75-110 μm in vivo; 2) different number of buccal cirri, *K. dieckmanni* 5-6 but *K. paradiackmanni* 5-8; 3) different number of frontal cirri, *K. dieckmanni* 5 but *K. paradiackmanni* 6-8; 4) different number and patterns of frontoventral cirri rows, *K. dieckmanni* 2 (3?) rows arranged as 4-6 (4-6-4?) from left to right; but *K. paradiackmanni* 3-4 rows, if 3 rows, 3-4-5 or 3-4-4 cirri arranged from the left row to the right one; if 4 rows, 3-4-5-5 or 2-3-3-4 arranged; 5) different number of transverse cirri, *K. dieckmanni* 4-7 but *K. paradiackmanni* 5-9; 6) different number of cirri in ventral rows, *K. dieckmanni* with 54 in the left ventral row and 50 in the right one, but *K. paradiackmanni* with 41 in the left ventral row and 48 in the right one in average; 7) different position relation between paroral and endoral membrane, paroral parallel to endoral in *K. dieckmanni*, but paroral crossed over endoral about its anterior or posterior 1/3 in *K. paradiackmanni* (Table 3).

Table 3. Differences between characteristics of *K. dieckmanni* and *K. paradiackmanni* sp. nov.

Characteristics	<i>K. dieckmanni</i>	<i>K. paradiackmanni</i> sp. nov.
Body size (μm)	165-245 $\times$ 48-91	180-320 $\times$ 75-110
BC, number	5-6	5-8
FC, number	5	6-8
FVC, rows	2 (3?)	3-4
FVC, rows and patterns (from left to right)	4, 6 (4, 6, 4?)	3-4-5 or 3-4-4, or 3-4-5-5 or 2-3-3-4
Cirri in left ventral row, number	54	41
Cirri in right ventral row, number	50	48
TC, number	4-7	5-9
Position relation between paroral and endoral	Paroral parallel to endoral	Paroral crossed over endoral about its anterior or posterior 1/3

BC-buccal cirri; FC-frontal cirri; FVC-frontoventral cirri; TC-transverse cirri.

Compared with other congeners, this new species can be distinguished by its body size, number of buccal cirri, number of frontal cirri, frontoventral cirri rows number and pattern, number of ventral cirri row, number of transverse cirri and especially position relation between paroral and endoral membranes.

Pseudochilodonopsis polyvacuolata Foissner and Didier, 1981 (Figs. 14-17)

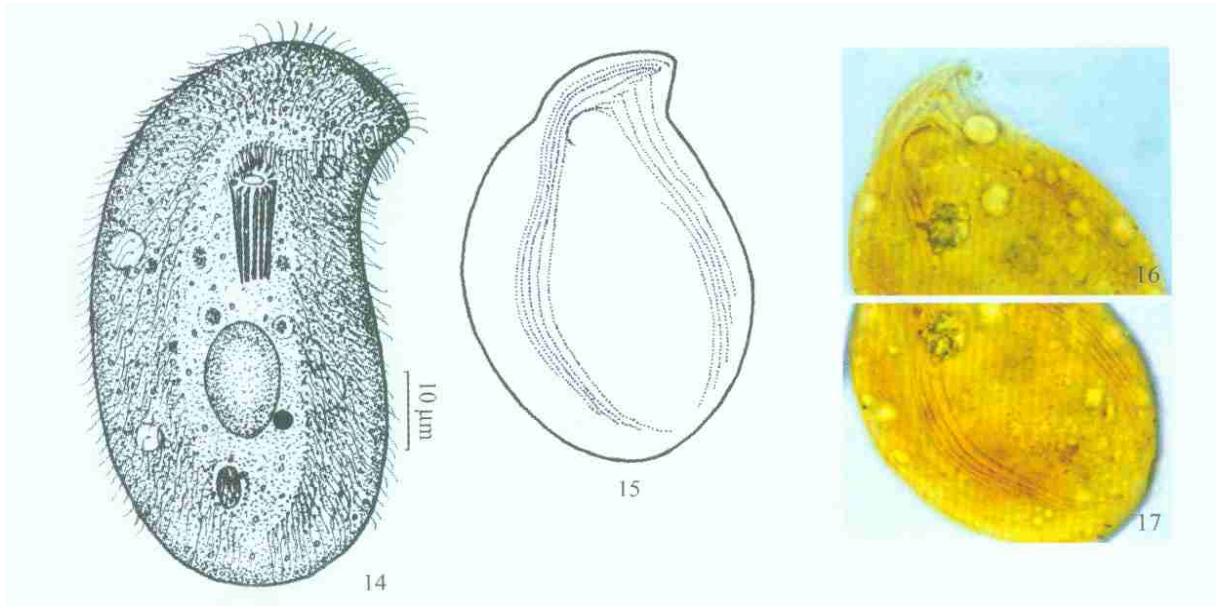
Size in vivo 70-80 μm $\times$ 35-40 μm (n = 7); acontractile, oviform to reniform with anterior slightly narrowed and bent to left forming a distinct rostrum, and posterior slightly broadened and posterior end broadly rounded; dorsoventrally flattened, ventral nearly plain, dorsal convex.

Eleven rows of ventral cilia with 6 rows on the left and 5 rows on the right and a nonciliated area without kineties below oral region; among the left rows, the

inner two started hypostomally and terminated posteriorly, and the others started from preoral suture and terminated medially, postmedially or posteriorly respectively; all the right rows started from preoral suture and terminated posteriorly; the left and right rows disjoined posteriorly; preoral kinety segmented into 4 segments and arranged along preoral suture; single ellipsoidal macronucleus vertical postequatorially slightly right of midline; single micronucleus close to the left lower of macronucleus. 4 contractile vacuoles, two of which arranged on the left and the other two on the right.

Horizontal elliptical cytostome in centre of anterior quarter of ventral surface; oral apparatus consisted of a basket of trichites.

Remark. The species was named by Foissner and Didier in 1981 and differed from its congeners by the increased number of contractile vacuoles, preoral kineties



Figs 14-17. Morphology of *Pseudochilodonopsis polyvacuolata*. 14. Ventral view in vivo. 15-17. Ventral view after protargol impregnation.

and somatic kinetics in the right ciliary field (Foissner, 1997); our population in conformity to original description.

Tetrahymena pyriformis (Ehrenberg, 1830) Lwoff, 1947 (Figs. 18-21)

Size in vivo $55-65\ \mu\text{m} \times 30-40\ \mu\text{m}$ ($n=9$); oviform or pyriform with anterior end bluntly pointed and posterior end broadly rounded; bilateral symmetric; 20 meridional rows of dense and uniform somatic cilia; preoral suture distinct; single spherical macronucleus equatorial; single more or less spherical micronucleus left anterior to macronucleus; single terminal contractile vacuole.

Vertical oral region in centre of anterior quarter to one third of ventral surface; oral apparatus consisted of three membranelles on the left and one C-shaped undulating membrane on the right.

Remark. *T. pyriformis* was a common species in freshwater and never reported in soil habitat before. Foissner (1987) considered that *T. pyriformis* (including all species of *T. pyriformis* complex) could only exist in freshwater because it (they) could not encyst. However, *T. pyriformis* was identified from several soil samples collected in the Scenic Spots and Historic Sites of Maijishan and Xinglongshan National Nature Reserve, Gansu.

Forming cysts was one way of physiological adaptations of soil ciliates to soil environment and was very important for ciliates to survive under unfavorable soil environmental conditions, especially poor water conditions. According to statistics, over 95 % of species of soil ciliates could form cysts (Foissner, 1987), which meant that there remained about 5 % of species either

could not form cysts or could form cysts which were not detected. Another way of physiological adaptations of soil ciliates was anabiosis (cryptobiosis, anhydrobiosis) (Foissner, 1987) which made organisms resist drying without forming cysts. It should be further studied that the existence of *T. pyriformis* in soils was either because it could form cysts which were not detected or because it possessed anabiosis or other unknown adaptation mechanism.

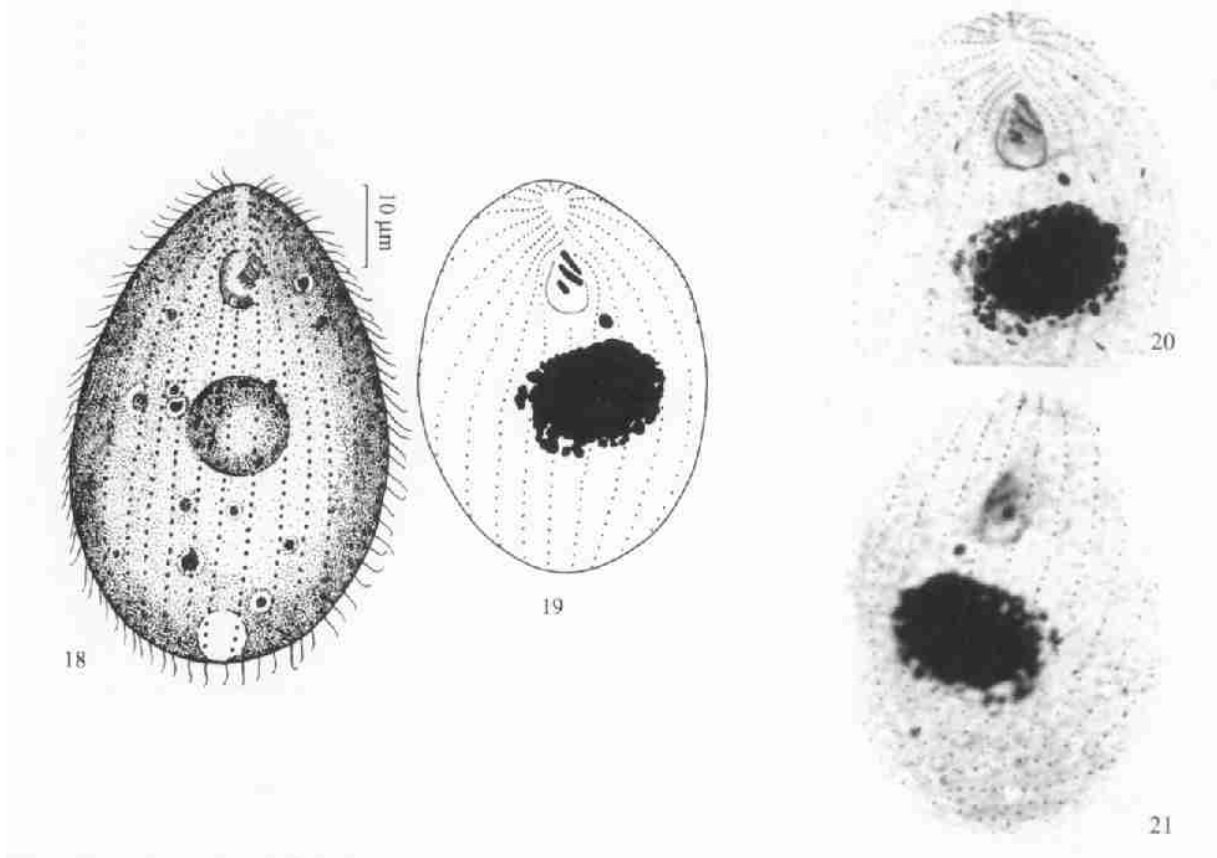
Sterkiella cavicola (Kahl, 1935) Foissner et al., 1991 (Figs. 22-32)

Size in vivo $150-168\ \mu\text{m} \times 68-75\ \mu\text{m}$ ($n=7$); flexible, oviform or marginals almost in parallel, with both ends more or less narrowly rounded; dorsoventrally flattened, ventral nearly plain, dorsal convex; 4 more or less spherical macronuclei one after another in the middle portion of cell obviously left of midline; 4 micronuclei clinged separately to the 4 macronuclei; colorless cytoplasm; contractile vacuole distinctly above midbody significantly left of midline.

3 frontal cirri; 1 buccal cirrus; 5 frontoventral cirri; 5 ventral cirri; 1 pretransverse ventral cirrus; 5 transverse cirri; 3 caudal cirri; one left and one right row of marginal cirri, the right one extended on dorsal side posteriorly; dorsal bristles arranged in 6 rows, among which the leftmost and the rightmost terminated at anterior quarter to half of dorsal surface, others terminated posteriorly.

AZM with 32-36 membranelles occupied about $1/3-1/2$ of body length; paroral and endoral membrane curved with the former crossed over the latter about its anterior $1/3$.

Remark. The species was identified first by Maupas



Figs. 18-21. Morphology of *Tetrahymena pyriformis*. 18. Ventral view in vivo. 19-20. Ventral view after protargol impregnation to show oral and nuclear apparatus. 21. Dorsal view after protargol impregnation showing somatic kineties and nuclear apparatus.

in 1888; unfortunately, it was misidentified as *Onychodromus grandis*; Kahl transferred the species to genus *Stylonychia* in 1932; furthermore, he classified *Stylonychia* as subgenus of *Oxytricha* in 1935 so that the species was named as *Oxytricha grandis* and then *Oxytricha cavicola*; afterwards, the name of the species was changed several times (Gelei & Szabadosz, 1950; Grolière, 1969; Grolière 1970; Borror, 1972; Matsusaka et al., 1984; Berger & Foissner, 1987) till the species was combined as *Sterkiella cavicola* by Foissner in 1991 (Foissner et al., 1991).

The individuals of this species observed by us were obviously smaller than all individuals described by the previous researchers (Kahl, 1935; Gelei & Szabadosz, 1950; Grolière, 1969; Berger & Foissner, 1987; Shin & Kim, 1994); besides, Berger (1999) considered that there invariably were 4 frontoventral cirri in the species, but 5 frontoventral cirri were observed in our observation.

Cyrtohymena tetracirrata (Gellét, 1942) Foissner, 1989 (Figs. 33-36)

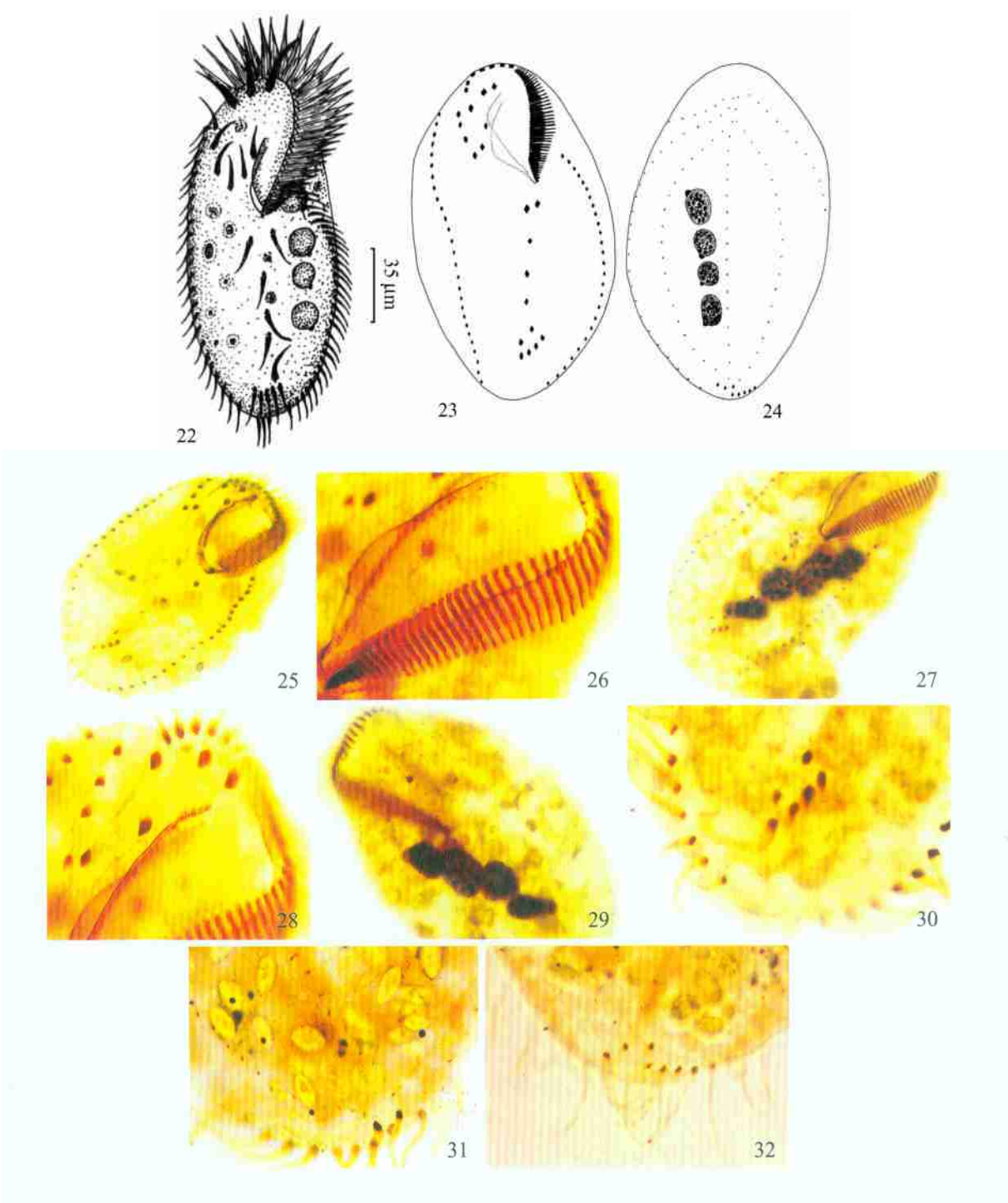
Size in vivo 140-155 μm $\times$ 50-60 μm ($n = 10$); rigid, elongated elliptical with left and right margin nearly straightened and posterior end broadly rounded; dorsoventrally flattened, ventral nearly plain, dorsal

slightly convex; cytoplasm packed with food granules, crystals, bacteria and symbiotic phytoflagellates; 2 elongated elliptical macronuclei one after another in the middle portion of cell slightly left of midline; 2 micronuclei clinged separately to the left of the 2 macronuclei; contractile vacuole just above equatorial slightly left of midline; rapid locomotion.

4 thick and strong frontal cirri; 3 frontoventral cirri arranged in V-shaped on the right of endoral membrane; 1 buccal cirrus; 3 ventral cirri; 2 pretransverse ventral cirri; 4 transverse cirri; 3 caudal cirri; one left row of marginal cirri with 19-26 cirri and one right row with 18-25; dorsal bristles arranged in 6 rows, among which the 2 rows on the right shortened and terminated at anterior quarter to one third of dorsal surface, others terminated posteriorly.

AZM with 35-37 membranelles occupied about 1/3-1/2 of body length; paroral membrane strongly curved and endoral membrane more or less curved with the former crossed over the latter about its anterior 1/2.

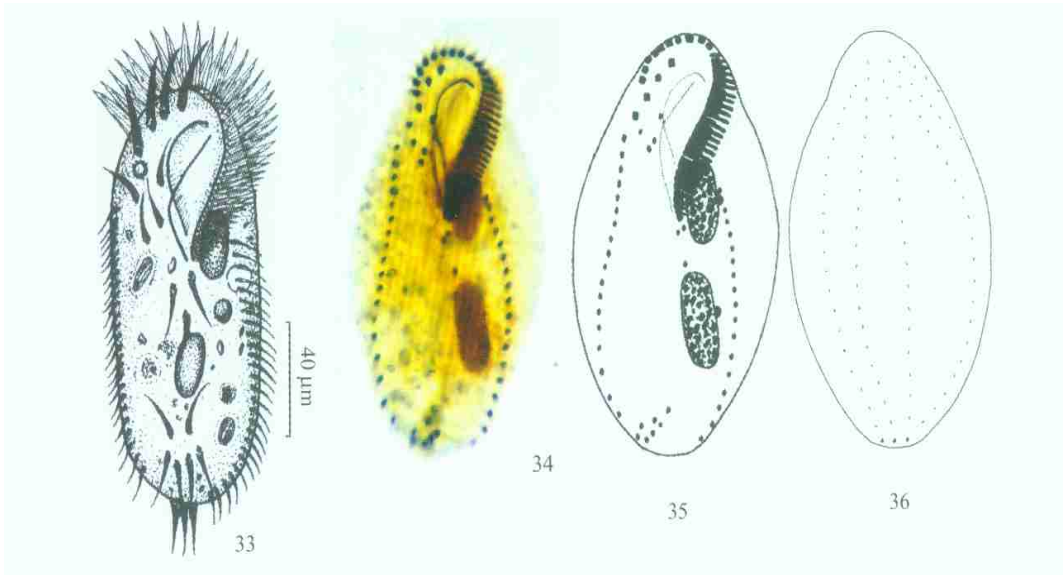
Remark. *C. tetracirrata*, a rigid terrestrial species with a typical *Oxytricha* ciliary pattern, but consistently with 4 transverse cirri, was originally described by Gellét (1942) with the name of *Steinia tetracirrata* (Song, 2004). It was transferred to *Oxytricha*



Figs. 22-32. *Sterkiella caviola*. 22. Ventral view in vivo. 23, 25-28, 30. Ventral view after protargol impregnation (26. Showing AZM and paroral and endoral membrane. 27. Showing macronuclei. 28. Showing FC, FVC, BC and paroral and endoral membrane. 30. Showing TC). 24, 29, 31-32. Dorsal view after protargol impregnation (29. Showing dorsal bristles and macronuclei. 31. Showing food vacuoles. 32. Showing CC and the posterior of right row of marginal cirri extended on dorsal side).

with the name *Oxytricha tetracirrata* by Borror (1972) and to *Steinia* again with the original name *Steinia tetracirrata* by Berger & Foissner (1987) ; afterwards , the species was transferred to *Cyrtohymena* by Foissner (1989) because its undulating membrane was curved as in *C. muscorum* , the type of the genus.

The morphogenesis of *C. tetracirrata* was studied by Song in 2004 (Song , 2004) , but the individuals he investigated were collected from South Africa. There was no report about the species domestically , our population slightly smaller than that reported by Berger (1999) , which is 150-170 µm ×60-70 µm but longer than Gell et



Figs. 33-36. *Cyrtolymna tetracirrata*. 33. Ventral view in vivo. 34-35. Ventral view after protargol impregnation. 36. Dorsal view after protargol impregnation.

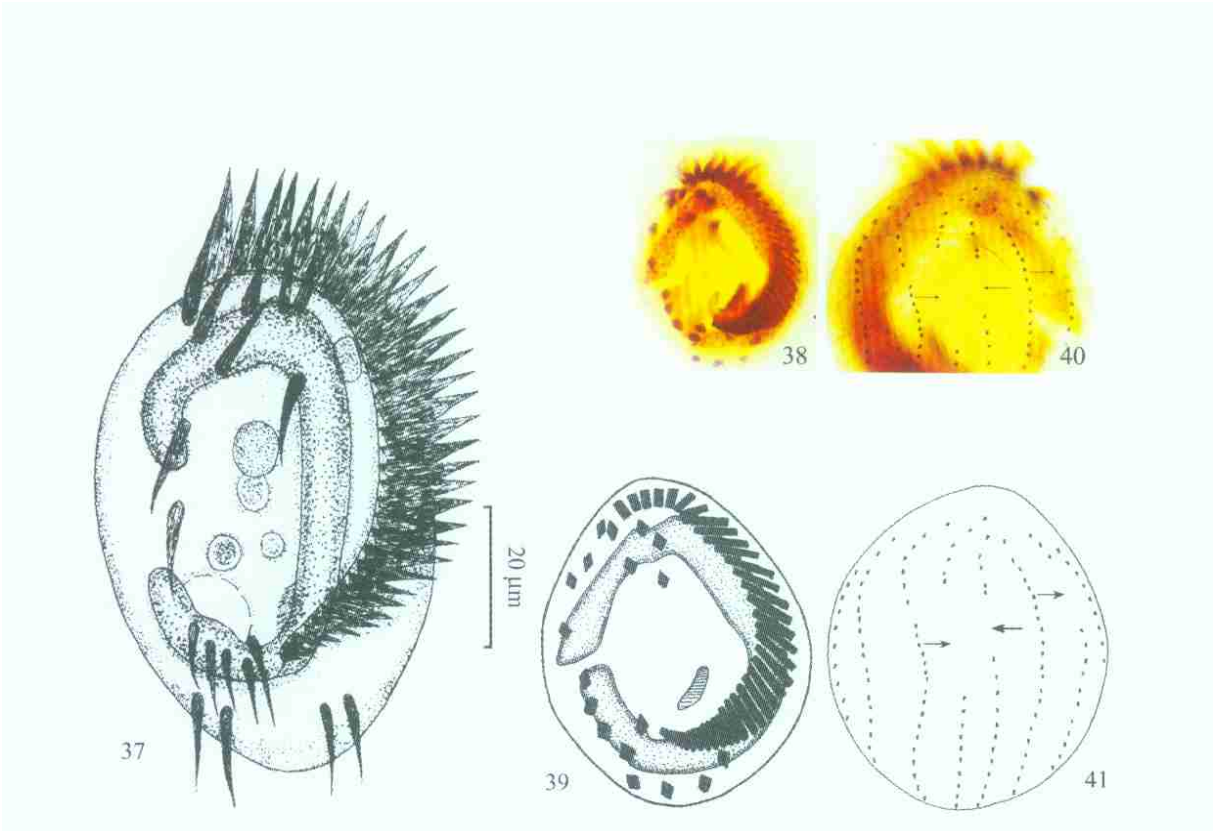
(1942 , 1956) , which is 90-110 μm long.

Euplotes elegans Kahl , 1932 (Figs. 37-41)

Size in vivo 70-80 μm ×45-50 μm (n = 10) ; rigid , obovate with anterior slightly broadened and posterior slightly narrowed , and anterior end more or less broadly rounded and posterior end more or less narrowly

rounded; vertical horseshoe-shaped macronucleus; 1 micronucleus clinged to left anterior to macronucleus; contractile vacuole in posterior 1/3 of cell , slightly right of midline.

9 frontoventral cirri; 5 transverse cirri; 4 caudal cirri; dorsal bristles arranged in 8 rows , among which the kinetosomes of the forth , fifth and seventh rows



Figs. 37-41. *Euplotes elegans*. 37. Ventral view in vivo. 38-39. Ventral view after protargol impregnation. 40-41. Dorsal view after protargol impregnation , arrows marked absence of kinetosomes.

(from left to right) absent in the middle portion.

AZM with 36-39 membranelles occupied about 3/4 of body length; small undulating membrane on the right of the end of AZM.

Remark. *E. elegans* was originally reported by Kahl (1932). Tuffrau (1960) and Carter (1972) described it in detail. Also Kahl (1932) and Dragesco have described a form of this species, *E. elegans* f. *littoralis*, but this was different from *E. elegans*. Curds (1975) gave a revision on genus *Euplotes* and redescription on *E. elegans*; some individuals observed in our research differed from those described by the previous researchers by the kinetosomes of the forth, fifth and seventh rows (from left to right) absent in the middle portion.

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中国土壤纤毛虫一新种和五新纪录种 (原生动物, 纤毛门)

宁应之¹ 刘汉成¹ 刘 娜¹ 孙宏飞² 张 超¹ 杜海峰¹ 王红军¹ 马正学¹

1. 西北师范大学生命科学学院 兰州 730070

2. 齐齐哈尔市福拉尔基区第五中学 齐齐哈尔 161041

摘 要 记述了采自甘肃麦积山风景名胜区和兴隆山国家级自然保护区土壤中的纤毛虫, 包括 1 新种和中国 5 新纪录种: 拟迪氏角毛虫 *Keronopsis paradiackmanni* sp. nov., 多泡伪拟斜管虫 *Pseudochilodonopsis polyvacuolata*, 梨形四膜虫 *Tetrahymena pyriformis*, 穴居斯道克虫 *Sterkiella cavicola*, 四棘毛管膜虫 *Cyrtohymena tetracirrata* 和优雅游仆虫 *Euplotes elegans*。

拟迪氏角毛虫, 新种 *Keronopsis paradiackmanni* sp. nov. (图 1~9)

鉴别特征为: 活体长 180~320 μm , 体宽 75~110 μm ; 体

关键词 原生动物, 纤毛门, 新种, 新纪录种, 土壤纤毛虫, 中国.

中图分类号 Q959.116

呈长椭圆形, 前后两端钝圆; 3~4 个大核, 短椭圆形; 4 个小核, 近圆形; 伸缩泡 1 个; 虫体有 6~8 根前触毛; 5~8 根口触毛; 3~4 列前腹触毛; 其中左侧一列断裂两次, 终止于横触毛之前; 左右缘触毛各 1 列; 5~9 根横触毛; 未见尾触毛; 背部刚毛列有 3 列; AZM 由 43~50 片小膜组成, 约占体长 $1/4 \sim 1/3$; 口旁膜与口后膜呈弧形, 口后膜在口旁膜的前 $1/3$ 或后 $1/3$ 处交叉。其模式标本保存于西北师范大学生命科学学院生态学与生物多样性研究所。